




## RESEARCH ARTICLE

# The long term vaccine-induced anti-SARS-CoV-2 immune response is impaired in quantity and quality under TNF $\alpha$ blockade

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## Abstract

The humoral immune response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination in patients with chronic inflammatory disease (CID) declines more rapidly with tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) inhibition. Furthermore, the efficacy of current vaccines against Omicron variants of concern (VOC) including BA.2 is limited. Alterations within immune cell populations, changes in IgG affinity, and the ability to neutralize a pre-VOC strain and the BA.2 virus were investigated in these at-risk patients. Serum levels of anti-SARS-CoV-2 IgG, IgG avidity, and neutralizing antibodies (NA) were determined in anti-TNF- $\alpha$  patients ( $n = 10$ ) and

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controls ( $n = 24$  healthy individuals;  $n = 12$  patients under other disease-modifying antirheumatic drugs, oDMARD) before and after the second and third vaccination by ELISA, immunoblot and live virus neutralization assay. SARS-CoV-2-specific B- and T cell subsets were analysed by multicolor flow cytometry. Six months after the second vaccination, anti-SARS-CoV-2 IgG levels, IgG avidity and anti-pre-VOC NA titres were significantly reduced in anti-TNF- $\alpha$  recipients compared to controls (healthy individuals: avidity:  $p \leq 0.0001$ ; NA:  $p = 0.0347$ ; oDMARDs: avidity:  $p = 0.0012$ ; NA:  $p = 0.0293$ ). The number of plasma cells was increased in anti-TNF- $\alpha$  patients (Healthy individuals:  $p = 0.0344$ ; oDMARDs:  $p = 0.0254$ ), while the absolute number of SARS-CoV-2-specific plasma cells 7 days after 2nd vaccination were comparable. Even after a third vaccination, these patients had lower anti-BA.2 NA titres compared to both other groups. We show a reduced SARS-CoV-2 neutralizing capacity in patients under TNF- $\alpha$  blockade. In this cohort, the plasma cell response appears to be less specific and shows stronger bystander activation. While these effects were observable after the first two vaccinations and with older VOC, the differences in responses to BA.2 were enhanced.

#### KEYWORDS

autoimmune diseases, COVID-19, tumor necrosis factor inhibitors, vaccination

## 1 | INTRODUCTION

The current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic poses a particular challenge for patients with chronic inflammatory disease (CID) receiving immunosuppressive therapies. For example, certain immunosuppressive therapies/pharmaceuticals (e.g., B cell depleting therapies, antimetabolites such as methotrexate, high-dose corticosteroids) are known to interfere with SARS-CoV-2 vaccine efficacy.<sup>1</sup> However, long-term data from this population on immune response to the vaccines are lacking.

Previously, we found that CID patients under tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) inhibiting therapy initially showed a largely normal, albeit slightly delayed, immune response to SARS-CoV-2 mRNA vaccines which was followed by a rapid decline of anti-spike (S) and virus-neutralizing antibody (NA) levels compared to patients receiving other disease-modifying antirheumatic drugs (oDMARDs) and healthy controls.<sup>2</sup> While the difference in anti-S antibody levels was marginal at Day 7 and absent at Day 14 after the second vaccination, these patients had significantly lower anti-S IgG levels 6 months after vaccination. Moreover, the neutralizing capacity of serum in CID patients treated with TNF $\alpha$  inhibitors was dramatically reduced at the sixth month after vaccination, as shown by a surrogate neutralization assay.<sup>3</sup> This impairment of adaptive immunity during anti-TNF- $\alpha$  treatment has also been confirmed by other research groups, including live virus neutralization data using the Delta variant of concern (VOC) as antigen.<sup>4,5</sup> Compared with healthy controls, anti-S IgA levels were decreased in CID patients at all time points after vaccination, suggesting impaired mucosal immunity.<sup>3</sup> It remains unclear what biological

mechanisms lead to this impaired antibody response and whether these differences indicate generally lower immunity after vaccination compared with controls. The relationship between B cells and T cells during SARS-CoV-2 vaccination is not fully understood, as humoral and T cell immunity appear to depend on B cell counts before vaccination.<sup>6</sup> In addition, data from immunocompromised kidney transplant patients show that T cell activity after vaccination correlates with the magnitude of the antibody response,<sup>7</sup> while high T cell activity has been observed in B cell-depleted patients after immunization.<sup>8</sup>

Sera from vaccinated healthy individuals show only limited neutralization capacity against Omicron (B.1.1.529) VOC.<sup>9,10</sup> This variant, consisting of several sublineages, including BA.1 and BA.2, is considered a separate serotype that is antigenically distinct from the original Wuhan strain (designated here as wild-type, wt, or pre-VOC) and other VOCs.<sup>11</sup> The marked immune escape of BA.1 and BA.2 and the importance of booster vaccination for the development of NA against both sublineages have recently been demonstrated,<sup>10</sup> especially the need of mRNA boost immunizations for persons vaccinated with inactivated viruses.<sup>12</sup>

Only limited data are available on the persistence of NA against various SARS-CoV-2 lineages (including Omicron) in CID patients receiving anti-TNF- $\alpha$  therapy after double vaccination. Virtually no data are available on the development of binding strength (avidity) of vaccine-induced IgG antibodies, which is considered an expression of their maturity and optimal epitope binding,<sup>13,14</sup> for this group of patients, nor are there any data on the development of cellular immunity.

The aim of this study is to clarify the influence of immunosuppressive therapy on the development of adaptive immunity after SARS-CoV-2

vaccination. To this end, the quality and quantity of SARS-CoV-2-specific B cells, plasmablasts, T cells, and antibodies were measured at different time points after the second vaccination in patients on TNF alpha blockade, patients on other DMARDs as well as healthy controls. We here report for the first time differential development of anti-BA.2 NAs after a third dose of vaccine in the three cohorts.

## 2 | METHODS

### 2.1 | Patient recruitment and biosampling

The study was approved by the ethics committee of the Christian-Albrecht University Kiel (D409/21). Recruitment of patients and repetitive biosampling was performed as previously described.<sup>2</sup> The SAVE-CID cohort consists of 47 healthcare workers and other risk groups who received their first SARS-CoV-2 vaccination in January 2021 followed by a second vaccination 5 or 3 weeks later. Samples taken 7 days after the third vaccination were also examined in 12 patients. All patients received BNT162b2 (Comirnaty, Pfizer/BioNTech) or mRNA-1273 (Spikevax, Moderna). Biosampling and data acquisition as well as data on antibody concentration, surrogate neutralization data and clinical characterization of this cohort has already been published.<sup>2,3</sup> The patient groups were age and gender-matched resulting in mean ages of 43 (TNF- $\alpha$  inhibitor, median: 42.5), 41.25 (Healthy Control, median: 39), and 41.63 (oDMARDs, median: 46).

### 2.2 | Production of SARS-CoV-2 S1 proteins

S1 domain of the S protein (GenBank: MN908947) with different tags were baculovirus-free produced in High Five cells (Thermo Fisher Scientific) by transient transfection as previously described.<sup>15,16</sup> Protein purification was performed depending 1 or 5 ml column on Äkta go (Cytiva), Äkta Pure (Cytiva), or Profina System (BIO-RAD). HiTrap Fibro Prisma (Cytiva) was used as resins for Protein A purification (Fc-tagged proteins). For His-tag purification of insect cell supernatant HisTrapexcel column (Cytiva) was used. All purifications were performed according to the manufacturer's manual. S1-HIS was further purified by SEC by a 16/600 Superdex 200 kDa pg (Cytiva).

### 2.3 | Isolation of peripheral blood mononuclear cells (PBMC) and flow cytometry

PBMCs from EDTA blood were isolated within 3 h of blood collection by density gradient centrifugation (Bicoll, Bio& SELL GmbH). Afterward,  $4 \times 10^6$  PBMCs were incubated with his-tagged S1 protein (own protein or Euroimmun).<sup>9</sup> PBMCs were then stained with pre-mixed antibodies (CD19-PerCP-Vio-700 (REA657, Miltenyi Biotec), CD20-PE-Cio770 (REA780, Miltenyi Biotec), CD3-Pacific Blue (OKT3, Biolegend), CD14 Pacific Blue (M5E2, Biolegend), CD27-APC (M-T271, Biolegend), anti-HIS-PE (JO95-G46, Biolegend),

Biolegend), HLA-DR-VioGreen (REA805, Miltenyi Biotec), CD138-BV605 MI15, Biolegend)) and analysed using a MacsQuant 16 Cytometer (Miltenyi Biotec). Secondary staining using AF-700 coupled S1-fc protein were used as gating and staining control to exclude false positive events (see Figures S1 and S2 for more information). For the calculation of immune cells per blood volume, 50  $\mu$ L of whole blood was stained, (CD3-Pacific Blue (Biolegend), CD14-FITC (REA599, Miltenyi Biotec), CD4-PE (Vit4, Miltenyi Biotec), CD19-PerCP-Vio700 and CD45-APC-Vio770 (H130, Biolegend)), lysed, (Red blood lysis, BD) and measured on a MACSQuant 16 (Miltenyi Biotec). The cell counts per 50  $\mu$ L blood for each sample were used to calculate all other cell counts from the PBMC staining. Staining and measurement were performed in PBS containing 1% BSA, 0.5% EDTA, and 0.1% sodium azide.

### 2.4 | Measurement of antibody-secreting cells by three-color fluorospot

PBMCs were isolated as described above and different cell numbers were incubated for 3 h in a 96-well 0.45  $\mu$ M PVDF Immobilon-FL membrane plate (Merck Millipore) which was pre-coated with SARS-CoV-2 protein or FAB2-fragments against Immunoglobulins A, M (both Southern Biotech), G (Jackson ImmunoResearch) and blocked with 15% FCS in PBS. After three washing steps with deionized water, the wells were stained using IgA-FITC, IgG-FC-AF555, and IgM-AF647 (all Southern Biotech). Measurement was performed on a Bioreader 6000Fb equipped with Eazyreader software (Bio-SYS).

### 2.5 | Antigen-reactive T cell enrichment (ARTE)

The ARTE was performed as previously described.<sup>17–20</sup> In brief,  $0.5–1 \times 10^7$  PBMCs were stimulated for 7 h with 0.5  $\mu$ g/peptide/ml SARS-CoV-2 S peptide pool (JPT) in presence of 1  $\mu$ g/ml CD40 and 1  $\mu$ g/ml CD28 pure antibody (both Miltenyi Biotec). 1  $\mu$ g/ml Brefeldin A (Sigma Aldrich) was added for the last 2 h. Cells were magnetically isolated using the CD154 MicroBead Kit (Miltenyi Biotec). After surface staining with CD4-APC-Vio770 (M-T466), CD8-VioGreen (REA734), CD14-VioGreen (REA599), CD20-VioGreen (LT20) (all Miltenyi Biotec), CD45RA-PE-Cy5 (HI100), PD-1 Brilliant Violet 605 (EH12.2H7), CCR7-Brilliant-Violet-785 (G043H7) (all BioLegend), cells were fixed, permeabilized and stained intracellular with CD154-FITC (REA238), IL-21-PE (REA1039) (both Miltenyi Biotec), IFN- $\gamma$ -PerCP-Cy5.5 (4S.B3), TNF- $\alpha$ -Brilliant-Violet-650 (MAb11), IL-10-PE-Dazzle (JES3-9D7) (all BioLegend), IL-2-BV711 (5344.111), and Ki-67-Alexa Fluor 700 (B56) (both BD Biosciences). Viability 405/520 Fixable Dye (Miltenyi Biotec) was used to exclude dead cells. Data were acquired on a LSR Fortessa (BD Bioscience, San Jose, CA, USA). CD154<sup>+</sup> background cells enriched from the non-stimulated control were subtracted and frequencies of antigen-specific T cells were determined based on CD154<sup>+</sup> T cells after enrichment, normalized to the total number of CD4<sup>+</sup> T cells applied on the column.

## 2.6 | Binding strength (avidity) of SARS-CoV-2 IgG antibodies

The IgG avidity was assessed with the *recomLine* SARS-CoV-2 IgG assay on a Dynablot Plus system together with a BLOTrix reader and the *recomScan* software (all from Mikrogen GmbH) as reported previously.<sup>21</sup> This immunoblot consists of a nitrocellulose strip separately carrying recombinant nucleocapsid protein (NP) and the S1- and RBD-subunits of the S protein. Binding of IgG to these SARS-CoV-2 antigens in the presence or absence of avidity reagent was automatically measured and assigned to four categories: no avidity detectable (=0), low avidity (=1), intermediate avidity (=2), and high (=3) avidity.<sup>22</sup>

## 2.7 | Measurement of neutralizing antibodies against a pre-VOC strain and a BA.2 strain

Sera were tested in triplicate using a Vero cell-based live virus neutralization test (cVNT) in 96-well format under biosafety level 3 conditions, as previously reported.<sup>10,22</sup> In brief, sera were diluted 1:10 to 1:1280 in cell culture medium free of fetal calf serum. As antigens for the cVNT, we used either 50 plaque-forming units per well of a B.1 strain (pre-VOC of 2020) or an Omicron BA.2 strain, which we had previously isolated<sup>23</sup> and characterized by whole-genome sequencing.<sup>10,23</sup> After 4 (pre-VOC) or 6 (BA.2) days of incubation, cells were fixed by addition of paraformaldehyde and stained with an aqueous crystal violet methanol solution. Serum dilutions (titers) > 1:10 that prevented the formation of a cytopathic effect in ≥2 wells were considered to contain neutralizing antibodies (NA); if no exact titers could be given, the geometric mean of the two adjacent titers was calculated.<sup>10,22</sup>

## 2.8 | Data analysis and statistics

Flow cytometry data was analysed using FlowJo v10 (BD Bioscience). Statistical analyses and graphs were prepared using RStudio (version 2022.02.0 + 443) and Prism 8 (GraphPad Software, LLC). For analysis of differences between the groups, Kruskal-Wallis Test and nonparametric pairwise comparisons were performed.

## 3 | RESULTS

### 3.1 | Anti-S-IgG-antibody concentrations, IgG avidity, and neutralization efficacy decrease 6 months after vaccination

Under TNF-α inhibitor therapy, anti-S-IgG levels after the second immunization were significantly lower than in patients receiving oDMARDs or in healthy controls (Figure 1A). None of the subjects showed anti-NP IgG reactivity, making infection breakthrough unlikely (data not shown). The IgG avidity and neutralization capacity

against the pre-VOC strain were high in all tested groups 14 days after second vaccination (avidity: median avidity index (MAI) = 3; NA: geometric mean titers (GMT) = 1:98–1:234) (Figure 1B,C). Six months after second vaccination, IgG avidity and pre-VOC NA titers significantly decreased in TNF-α-inhibitor treated vaccinees ( $n = 8$ ; avidity: MAI = 1.25; NA: GMT = 1:2) compared to patients receiving oDMARDs ( $n = 7$ ; avidity: MAI = 3,  $p = 0.0012$ ; NA: GMT = 1:38,  $p = 0.0293$ ) and healthy controls ( $n = 12$ ; avidity: MAI = 3,  $p \leq 0.0001$ ; NA: GMT = 1:25,  $p = 0.0347$ ) (Figure 1B,C). Relative to the pre-VOC strain, anti-BA.2 NA titers were significantly lower than against the pre-VOC strain in all three groups at day 14 after the second vaccination (GMT = 1:2–1:4 vs. GMT = 1:98–1:234;  $p = 0.0001$ – $0.0072$ ) and were not detected after six months (GMT < 1:10, Figure 1D). At 7 days after the third vaccination, anti-BA.2 NAs were detectable in all subjects except patients taking a TNF-α blocker ( $n = 4$  per group; GMT = 1:62–1:95 vs. GMT = 1:3). At this time point, IgG avidity was high in all subjects except one patient receiving anti-TNF-α treatment.

### 3.2 | Plasma cell populations are altered in patients using TNF-α blockers

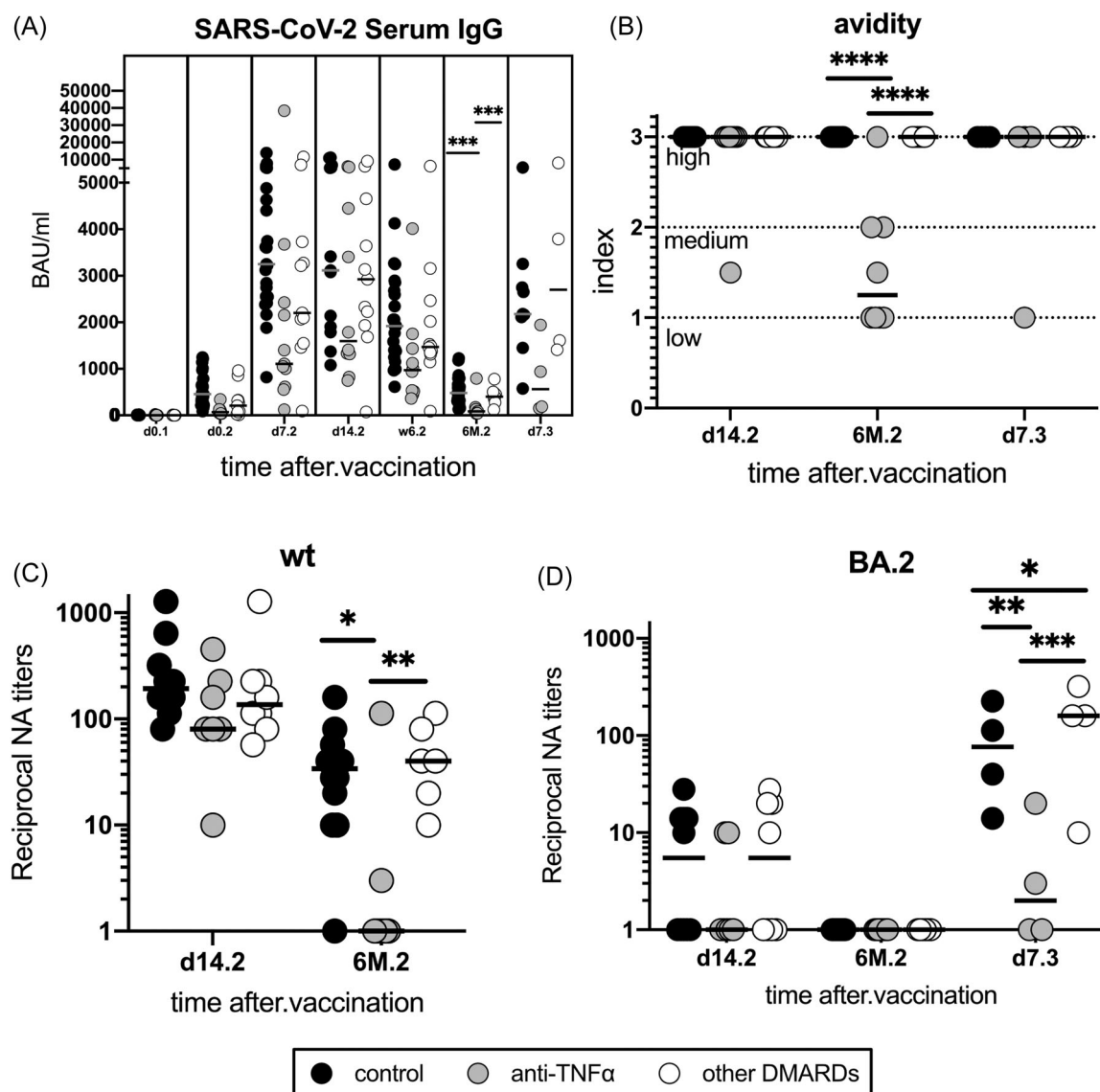
Patients under TNF-α inhibiting therapy showed higher numbers of plasmablasts in the peripheral blood 7 days after the second vaccination (median: 9.153 cells/μL) compared to patients receiving oDMARDs (median: 2.205 cells/μL,  $p = 0.0254$ ) (Figure 2A) and healthy controls (2.657 cells/μL,  $p = 0.0344$ ).

When comparing SARS-CoV-2 specific plasma cells at the same timepoint, no differences between patients on TNF-α blockade (median 0.295 cells/μL) and healthy controls (median 0.333 cells/μL) were detected, while patients treated with oDMARDs other than TNF-α inhibitors had significantly lower cell numbers compared with healthy controls (median: 0.204 cells/μL;  $p = 0.0015$ ) (Figure 2B).

Anti-TNF-α treated patients generally displayed more peripheral blood IgA plasma cells than controls and patients receiving oDMARDs (Figure 2C). This trend became significant on Day 14 after vaccination for patients under oDMARDs (median 0.404 cells/μL) and TNF-α blockers (median 1.818 cells/μL;  $p = 0.0397$ ).

With regard to SARS-CoV-2-specific IgA-plasma cells, counts were lower in the anti-TNF-α group on Day 7 after the second vaccination (median: 0.016 cells/μL) compared with healthy controls (median: 0.052 cells/μL;  $p = 0.0203$ ) and patients treated with oDMARDs (median: 0.035 cells/μL;  $p = 0.0299$ ) (Figure 1D).

Overall numbers of mature circulating CD138<sup>+</sup> plasma cells were higher in patients on TNF-α blockade (median: 0.101 cells/μL) than in healthy controls (median: 0.031 cells/μL;  $p = 0.0055$ ) and patients treated with oDMARDs (median: 0.019 cells/μL;  $p = 0.0015$ ) on Day 14 after second vaccination (Figure 2E). Differences at time points between the first and the second vaccination were not significant. We were not able to detect differences in the number of SARS-CoV-2 specific plasma cells. However, on Day 7 after second vaccination, healthy controls had higher numbers of circulating CD138<sup>+</sup> plasma



**FIGURE 1** (A) Patients using TNF- $\alpha$  blockers show reduced antibody serum levels, avidity and neutralization at different timepoints. Serum IgG antibody against SARS-CoV-2 Spike 1 subunit at different timepoints as single values per patient. Lines indicate the median. (B) IgG avidity indices of anti-S IgGs of SARS-CoV-2. (C) Neutralizing antibodies against the wild-type (wt) and (D) BA.2 variant of SARS-CoV-2 at 14 days and 6 months after the second vaccination. For BA.2 and IgG avidity, a time point 7 days after the third vaccination was added ( $n = 4$  per group); values are given as individual values and median. Statistical differences: Kruskal-Wallis test with Dunn's post hoc test, significant differences are indicated as \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

cells in the peripheral blood (median: 0.004 cells/ $\mu$ l) than patients using TNF- $\alpha$  inhibitors (median: 0.002 cells/ $\mu$ l;  $p = 0.0055$ ) or receiving oDMARDs (median: 0.001 cells/ $\mu$ l;  $p = 0.0026$ ) (Figure 2F).

### 3.3 | Anti-TNF- $\alpha$ treatment does not change the number of SARS-CoV-2 specific antibody-secreting cells

Relative to the other two sample groups, anti-TNF- $\alpha$  patients showed a pronounced increase in SARS-CoV-2 specific antibody-secreting cells (ASCs) of the IgM isotype at day 7 post second vaccination, while total

numbers of ASC of all other isotypes remained comparable between groups (Figure 3A). Analysing spot size in our Fluorospot assays as a surrogate for the amount of secreted antibody per cell, both patient groups generally displayed larger spot sizes suggesting increased antibody secretion per ASC (Figure 3B). These differences were not significant except for IgA ( $p = 0.0026$  for TNF vs. Healthy Control and  $p = 0.001$  for oDMARDs vs. Healthy Control). No differences were detected between the two patient groups. The number of ASCs correlated well with SARS-CoV-2 serum IgG levels and the number of SARS-CoV-2 positive plasmablasts at the same timepoint (Figure 3C,D). No antigen-specific plasma cells were detected in the blood of any participant before the first vaccination (data not shown).



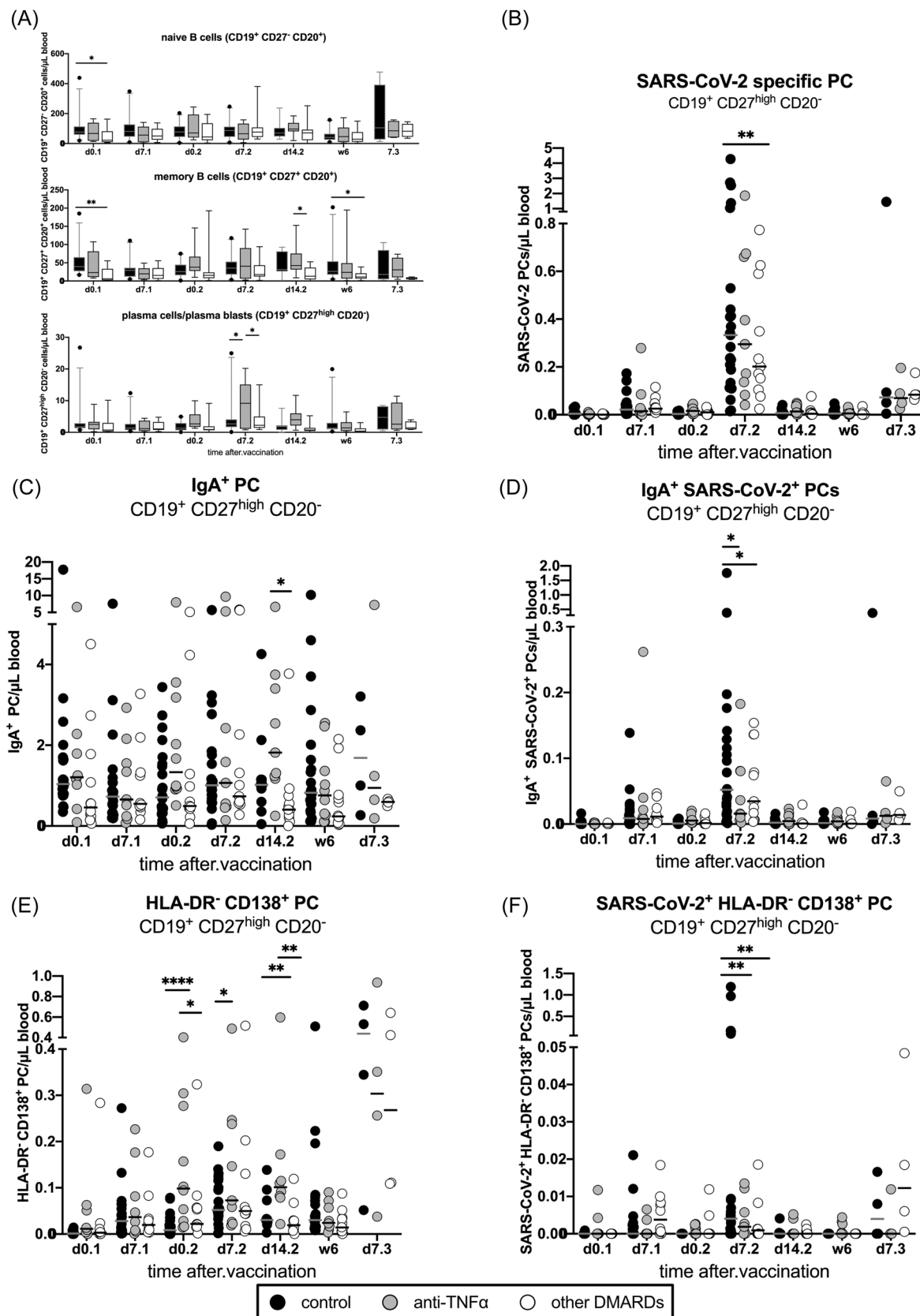
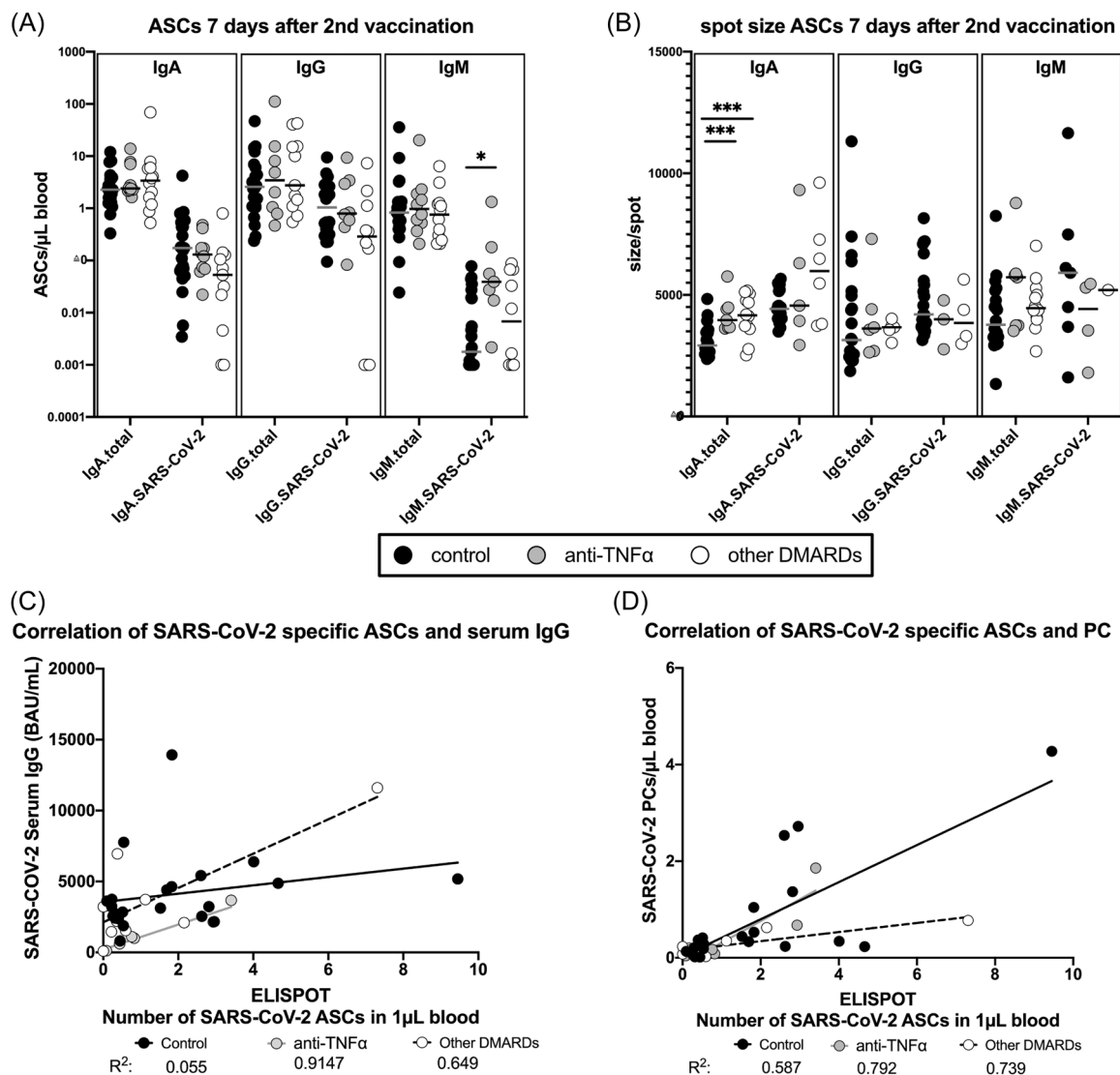


FIGURE 2 (See caption on next page)



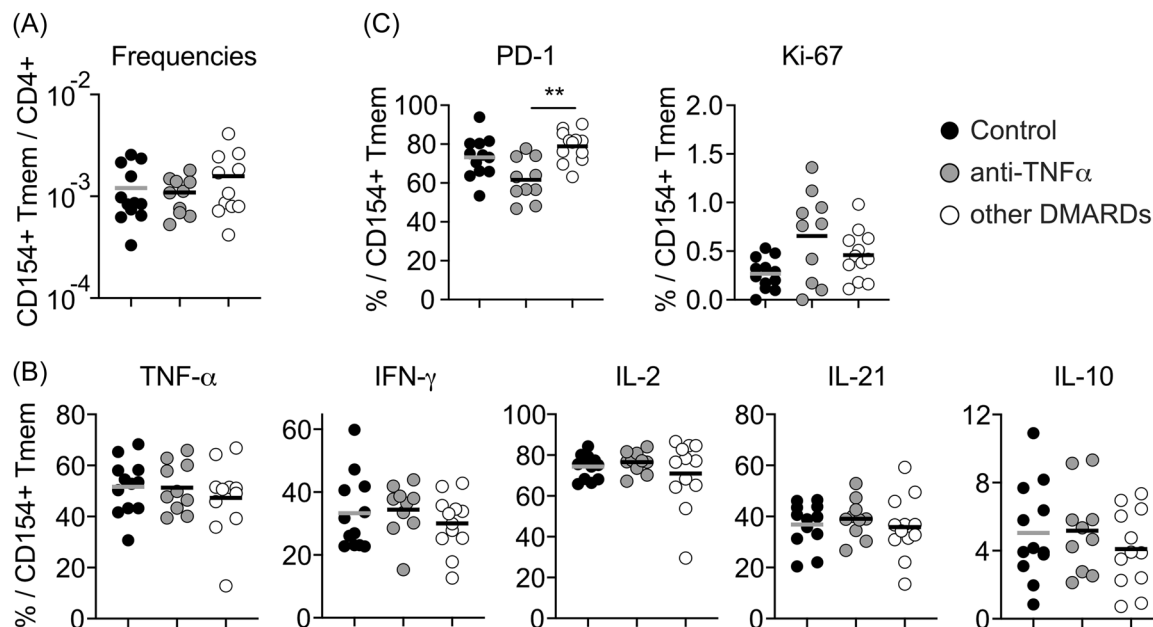
**FIGURE 3** Antibody secreting cells (ASC) measured in 3-color-Fluorospot 7 days after the second vaccination. See figure S3 for representative image. (A) Number of total and SARS-CoV-2 specific ASCs per µL blood. (B) Spot size distribution. Single points resemble the mean spot size per spot of one donor. Correlation (Pearson) of the number of SARS-CoV-2 specific ASCs (C) against SARS-CoV-2 specific plasmablasts in flow cytometry and (D) against SARS-CoV-2 serum IgG. Regression line in grey resembles TNF-α group and dashed line other DMARDs. TNF Statistical differences: Kruskal-Wallis test with Dunn's post hoc test, significant differences are indicated as \* $p < 0.05$ , \*\*\* $p < 0.001$ . SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNF-α, tumor necrosis factor-α.

### 3.4 | SARS-CoV-2 specific T cells show signs of delayed activation

SARS-CoV-2 S-specific CD4<sup>+</sup> T cells could be detected at similar frequencies in all groups after second vaccination (Figure 4A). We also observed no differences in cytokine

production (TNF-α, IFNγ, IL-2, IL-21, or IL-10) by the S-specific T cells between TNF-α patients, oDMARDs, and controls (Figure 4B). In contrast, TNF-α patients showed significantly lower expression of PD-1 and a trend towards increased levels of Ki-67, suggesting a delayed or still ongoing activation of these cells (Figure 4C).

**FIGURE 2** Patients using TNF-α blockers show higher numbers of overall plasmablasts and SARS-CoV-2 specific plasmablasts 7 days after the second vaccination. (A) Flow cytometry analysis of B cell subsets at different timepoints after a vaccination against SARS-CoV-2. Values are shown as cells per microlitre of blood. Boxes and whiskers indicate median and 95%CI. (B) SARS-CoV-2 specific plasmablasts. IgA<sup>+</sup> plasmablasts (C) and SARS-CoV-2 specific (D) IgA<sup>+</sup> positive plasmablasts. (E) and (F) CD138<sup>+</sup> unspecific and SARS-CoV-2 specific plasmablasts which resemble mature plasma cells. If not indicated otherwise, cell counts per microlitre blood are shown as single values with median. Statistical differences: Kruskal-Wallis test with Dunn's post hoc test, significant differences are indicated as \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.0001$ . SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNF-α, tumor necrosis factor-α.



**FIGURE 4** SARS-CoV-2 specific memory T cells in patients receiving TNF- $\alpha$  blockers show a delayed activation status. Antigen-reactive T cell enrichment was performed two months after the second vaccination. (A) Frequencies of spike-reactive CD154<sup>+</sup> memory cells within CD4<sup>+</sup> T cells. (B) Percentage of cytokine production within spike-reactive CD154<sup>+</sup> memory cells. (C) Percentage of PD-1 and Ki-67 positive cells within CD154<sup>+</sup> memory cells. Each dot represents one donor, lines indicate mean values. Statistical differences: Kruskal-Wallis test with Dunn's post hoc test, significant differences are indicated as \*\* $p < 0.01$ . SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

## 4 | DISCUSSION

To our knowledge, we present the first such comprehensive data on the longitudinal course of adaptive immunity in CID patients vaccinated against SARS-CoV-2 undergoing TNF- $\alpha$  blockade.

These patients show an altered immune response after vaccination relative to oDMARD patients and healthy controls in the absence of breakthrough infections, suggesting that such individuals should be monitored more closely for loss of SARS-CoV-2 immunity.

Most strikingly, the anti-TNF- $\alpha$  patients showed a decrease in IgG avidity and a greater loss of neutralizing capacity 6 months after vaccination. Moreover, they did not acquire sufficient NA response against BA.2 after a third vaccination. To our knowledge this is the first report concerning the long-term neutralization efficacy against BA.2 VOC within this patient population after vaccination. While the neutralization efficiency against the initial pre-VOC wt strain was marginally lower in anti-TNF- $\alpha$ -treated patients 14 days after second vaccination compared to the other groups, this difference was more pronounced against the BA.2 strain where overall low neutralization was detected. Six months after second vaccination, BA.2 NAs were not detected in any of the three study groups, suggesting that the S antigen of this VOC is relevantly different from that of wt and previous VOCs, consistent with recent studies.<sup>10,24</sup>

The loss of IgG avidity was unexpected as anti-TNF- $\alpha$  patients displayed similarly high IgG avidity as the other groups at Day 14 post second vaccination. Conversely, other studies have shown, that the avidity of anti-SARS-CoV-2 IgG increases during the subsequent

months after vaccination.<sup>13</sup> In line with these results, a recent publication has shown that three vaccinations with the wt S protein-based vaccines are required to achieve at all highly avid antibodies against the Omicron VOC.<sup>25</sup> This decline of avidity several months after the vaccination in anti-TNF- $\alpha$  patients has not been previously reported.

In addition, various changes in the plasmablast compartment of patients were observed in response to vaccination. Hence, patients using TNF inhibitors had higher numbers of plasma cells after the first vaccination (d0.2) relative to the two other groups. These differences increase after the second vaccination, suggesting a stronger immune reaction. The frequency of SARS-CoV-2 specific plasma cells within this population was decreased in the patient groups compared to controls. The absolute number of SARS-CoV-2 specific plasmablasts however was comparable to the other groups, suggesting that the immune reaction triggered under TNF- $\alpha$  therapy is more unspecific. The ASCs data follows these flow cytometric data with some differences: we could not observe differences in the number of total ASCs between groups, but cells from patients on TNF blockade showed a trend to produce more antibodies per cell (as defined by spot size). In addition, a trend was observed towards lower numbers of SARS-CoV-2 specific ASCs. The differences in comparison to the flow cytometry data can be explained by the method: While cells in flow cytometry are distinguished by cell surface markers—more or less specific for antibody-secreting cells, in fluorospot only functional antibody-secreting cells are measured.

Higher numbers of mature CD138<sup>+</sup> plasmablasts were detected within the non-SARS-CoV-2-specific plasma cells from anti-TNF- $\alpha$



patients. These cells are usually found in the bone marrow.<sup>26</sup> The difference in maturity between S-specific and nonspecific plasma cells may represent a reduced capacity to form de novo long-lived specific plasma cells under anti-TNF- $\alpha$  therapy, consistent with the rapid decline of antibody titers over time. However, assessment of these cells in the peripheral blood might not reflect their state in the bone marrow and our measurements after second vaccination may be too early for the detection of long-lived SARS-CoV-2-specific plasma cells. We also noted that a large proportion of these CD138<sup>+</sup> plasma cells expressed HLA-DR (Figure S4). These phenotypically mature (CD138<sup>+</sup>) and antigen presenting plasmablasts (HLA-DR) were also described in patients with severe COVID-19<sup>27</sup> their function remains unclear.

The diminished plasma cell responses which result in less avidity and neutralization capacity, could be, at least partly, a consequence of Tregs expressing more KI67, resembling a delayed, still activated T cell response.<sup>28</sup>

Interestingly, although broadly homogeneous, there were two outliers in our cohort of anti-TNF- $\alpha$  patients. One patient had consistently high antibody concentrations, high IgG avidity and NAs at 6 months. The other patient had consistently low values for all three parameters. These outliers show the importance of individual monitoring of patients at risk of reduced immunity after vaccination.

Our data demonstrate that TNF- $\alpha$  inhibitors affect the adaptive immune response after SARS-CoV-2 vaccination. This is reflected in the development of IgG avidity, virus neutralizing capacity, plasma cell, and T cell populations. We recommend regular measurement of anti-SARS-CoV-2 IgG levels and especially NA titers in these patients, as this group may benefit from early booster vaccination. To our knowledge, current commercial anti-SARS-CoV-2 antibody tests are based on antigens still derived from the wild type. However, if available, assays adapted to the currently circulating variants should preferably be used. In addition, the development of variant-specific surrogate neutralization tests would be desirable, as these, unlike live virus neutralization assays, can also be used in routine laboratories. CID patients on TNF- $\alpha$  inhibitor therapy who have no, or low detectable antibody levels should be particularly protected from COVID-19. Following SARS-CoV-2 infection, these patients might require close monitoring and early administration of monoclonal antibodies or antiviral medication that also cover currently circulating VOC. In addition, we recommend the use of a vaccine adapted to the current VOC as soon as it becomes available.

The mechanisms leading to decreased antibody response during immunosuppressive treatment need to be further explored to improve vaccine regimens for these high-risk patients.

## AUTHOR CONTRIBUTIONS

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## CONFLICTS OF INTEREST

BFH, PH, and SS received funding from Pfizer. The authors state no conflicts of interest in the context of this study.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ETHICS STATEMENT

The study was approved by the ethics committee of the Christian-Albrecht University Kiel (D409/21). All patients signed informed consent. The study is registered at DRKS (DRKS00024214).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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